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Impact of Protein Deficient Diets Supplemented with Methionine on Growth, Nutrient Utilization and Carcass Amino Acid Profile of African Catfish *Clarias Gariepinus* (Burchell, 1822)

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Abstract

Fish farmers often use practical diets deficient in proteins in order to minimize costs and maximize profits. This study was conducted to determine the effects of DL- Methionine supplementation in such diets on growth and carcass quality of African catfish *Clarias gariepinus.* Results showed that feeding fish with protein deficient diets retarded growth performance, reduced nutrients composition and increased the carcass lipid. However, supplementation of Met in the diets generally improved the fish growth performance, nutrient composition and enhanced the synthesis of other amino acids in the fish body. Similarly, supplementation of Met to protein deficient 35% crude protein (CP) diet raised the Met content of that diet that it produced fish with similar growth performance as those fed the control diets of 40 or 45% CP. The study also revealed no additional significant benefits by increasing the dietary protein level of the fish from 40 to 45%, thus confirming 40% protein as the requirement for the fish. However, 35% crude protein diet supplemented with 0.11% DL-Met was as efficient as a diet of 40% CP in supporting the growth performance and nutrient utilization and in regulating the carcass quality of the fish.

Keywords: Farm made Protein deficient diets, African catfish, Methionine.

Introduction

Global aquaculture is growing at more than 8% annually. Its contribution to world total fish production climbed steadily from 20.9 percent in 1995 to 32.4 percent in 2005 and 47 percent in 2010 (FAO, 2012). It attained all-time high in 2011, at 60 million tonnes (excluding aquatic plants and non-food products), with an estimated total value of US$119 billion (FAO, 2012). Fish meal is the major ingredient in commercial fish feeds, especially those used for rearing carnivorous species, such as catfishes, salmon, trout and many marine fishes (Sugiura et al. 2001). It has balanced amino acid profile which makes it suitable as animal protein in fish nutrition.

However, it is scarce and expensive, resulting in over 65% of the operating costs of aquaculture (Lovell 1989, Nwanna, 2002, Luo et al., 2004). Many of the popular aquaculture species including salmon, trouts, catfishes and tilapias are farmed on wild fish as protein in their diets, (fish eat fish) system. Presently, about 11 million tones of fish constituting 12% of the total haul from seas and rivers are used each year to feed the farmed fish. Meanwhile, it takes 2 to 5 kg of wild fish just to produce 1 kg of a farmed fish such as salmon (Kendall 2003).

Also in the recent time, climate change and unprecedented rain fall pattern and flooding have affected arable crops production negatively, resulting in scarcity of plant protein ingredients used in fish feed, thereby forcing the prices of available ones to double or triple especially in the tropical developing countries. Therefore, in the face of declining capture fisheries and a booming aquaculture industry, many fish farmers have started cutting the costs of feed production by preparing practical diets deficient in proteins. As protein is the most important factor affecting growth performance of fish (Luo et al., 2004), it becomes necessary to study and document the effects of low protein diets on the growth of fishes. This study therefore was aimed at investigating the effects of protein deficient diets on the growth, nutrient utilization and carcass quality of African catfish (*Clarias gariepinus*) and whether protein deficient diets supplemented with DL-Methionine could produce good growth performance as the diets sufficient in protein.

Materials and Methods

Experimental animals and deign

Three hundred apparently healthy fingerlings of *C. gariepinus* (2.40-2.50 g) were acclimatized to the laboratory conditions for 15 days using five 700 L capacity flow-through fibre-reinforced plastic tanks with the provision of continuous aeration. During acclimatization, the fish were fed with a fish meal-based formulated diet (400 g protein kg). Fish were weighed individually and two hundred and seventy uniform-sized healthy fish (2.40-2.50g) were equally distributed in six dietary treatment groups, T_1 (30% CP diet), T_2 (30% CP diet +0.11% DL-Met), T_3 (35% CP diet), T_4 (35% CP diet + 0.11% Met), T_5 (40% CP diet), T_6 (45% CP diet) each in triplicate and stocking density of 15 fish per tank in 150 L of rearing water, in a completely randomized design. The150 L capacity flow through glass tanks with a flow rate of 1.5 L min were used for rearing the fish. Tap water was used through out the experimental period. Treatments 5 and 6 served as the control as authors reported the protein requirement of the same size of *C. gariepinus* used in the present study to be either 40 or 45% (Fagbenro et al. 1992; Anyanwu 2000).

Feed preparation and feeding

In preparing the experimental diets, the required ingredients including fish meal, soybean meal, groundnut cake, maize, fish/vegetable oil, methionine, vitamin mineral premix, and carboxymethyl cellulose were procured (Table I). The major ingredients were blended into fine powder and sieved through a fine-meshed screen (0.5mm diameter) (Mohapatra et al. 2012). The required feed ingredients were mixed with carboxymethyl cellulose, vitamins and minerals and oil and thoroughly mixed together. Water was added and mixed with all the ingredients to make dough. Finally, the dough was pelletized using Hobart pelleting machine (Hobart Model 200, CA,USA) to get uniform size pellets (2 mm) and oven dried over night at 45^{o}C, the dried pellets were kept at -4^{o}C before use.

Proximate compositions of the six diets were determined according to AOAC (1995) (Table I), while gross energy of the diets was calculated according to NRC (2011), based on physiological values of 4.11, 5.64 and 9.44 kcal/g (17.2, 23.6, and 39.5 KJ/g) for carbohydrates, proteins, and lipids, respectively (Table 1).
The amino acids compositions of the diets (Table 2_b) were calculated from the feed nutrient composition tables of (NRC 2011), (Table 2a). All the treatment groups of fish were fed to apparent satiation thrice daily. Fish were weighed bi-weekly for calculation of the growth parameters.

At the end of the experiment which lasted for 70 days, fish were counted and weighed. The growth parameters and feed utilization indices were calculated as follows: Weight gain = Final wt. – initial wt. Specific growth rate (SGR) = 100 (Ln W_2 - Ln W_1)/T; where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days in the feeding period; Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g); Protein efficiency ratio (PER) = Weight gain (g)/Protein intake (g).

Carcass proximate, minerals and amino acids analyses

At the end of the experiment fish were not fed for 24h. The catfish were weighed and five fish from each tank was collected. A total of 15 fish from each treatment were killed by anaesthetic overdose (ethylene-glycol-monophenyl-ether) (Liebert et al., 2006), oven dried at 48°C (whole body) for 24h, blended into powder and kept at -4°C before use. Three replicates of the stored fish samples were analysed for proximate and minerals composition according to the standard methods of AOAC (1995).

Amino acids analyses

The stored fish carcass were blended into fine powder and sieved through a fine-meshed screen (0.5mm diameter) (Liebert et al 2006) before amino acids analyses. Then the amino acid analyses of triplicate samples of the fish were conducted by ion exchange chromatography (LC 3000; Biotronik, Eppendorf-Netheler-Hinz, Hamburg, Germany) following acid hydrolysis with and without an oxidation step for the sulphur containing amino acids.

Statistical analyses

Data were subjected to analyses of variance using SAS (2003)and comparison among treatment means at (P=0.05) was by Duncan's multiple range test.

Results

The proximate composition of the experimental diets (Table 1) showed closely related values according to dietary formulations. The protein contents of the diets increased accordingly resulting to marginal increases in the gross energy of the diets.

Table 2_a shows the amino acids contents of the major feed ingredients used in calculating the amino acids contents of the experimental diets as shown in Table 2_b, which shows increase in the amino acids contents of the diets with increasing dietary protein levels. From the dietary formulations, the major limiting amino acid is methionine. Therefore supplementation of 0.11% of DL-Met increased the Met level in diet 4 to 0.79%, which is close to 0.75% and 0.83% Met contents in the control diets 5 and 6.

The growth and nutrient utilization of the fish (Table 3) indicated that feeding the fish with 30 or 35% dietary protein retarded the mean weight gain of the fish significantly. However, addition of 0.11% DL-Met to the diet of 35% protein increased the Met to the level required by the fish resulting in similar (P>0.05) growth performance with the fish fed 40 or 45% CP diets.

The specific growth rate increased with increasing dietary protein levels or with the supplementation of DL-Met in the diets as there was no significant increase in the SGR of fish fed diets 4, 5 and 6. SGR of the fish fed diet 4 was also marginally higher than those of the fish fed diets 2 and 3, but significantly higher than that of the fish fed diet 1. The feed conversion ratio (FCR) and protein efficiency ratio (PER) improved with increasing dietary protein levels or with DL-Met supplementation but there were no significant differences in the FCR and PER of fish under the six different treatments.

Proximate composition of the fish carcass (Table 4) indicated that the protein and ash contents of the fish increased with increasing dietary protein levels or with DL-Met supplementation, but while there were no differences (P>0.05) in protein levels of fish in all the treatments, significant differences existed in the carcass ash. Fish fed diets 3, 4, 5 and 6 contained the same (P>0.05) quantity of ash which were higher (P<0.05) than those of the fish fed diets 1 and 2. Lipid contents of the fish showed a declining trend with increasing levels of protein in the diets. The protein and ash ratio (protein: ash) was calculated to test the hypothesis that they are constant. But the result did not have very clear interpretation, although there were no significant differences in the values.

Table 1. Gross composition of experimental diets (g kg^{-1} DM)

Ingredients	Diet 1 30% CP	Diet 2 30% CP+Met	Diet 3 35% CP	Diet 4 35% CP+Met	Diet 5 40% CP	Diet 6 45% CP
Fish meal	174.7	174.7	203.8	203.8	233	262.1
Soybean meal	190.5	190.5	221	221	254	285.1
Ground nut cake	210	210	246	246	280	316
Yellow maize	318.6	318.6	223	223	138	41.8
Fish/Veg oil	60.0	60.0	60.0	60.0	60.0	60.0
Vitamin-premix[1]	25.0	25.0	25.0	25.0	25.0	25.0
Carboxylmethyl cellulose	10.0	10.0	10.0	10.0	10.0	10.0
DL-methionine	0.0	1,10	0.0	1,10	0.0	0.0
Proximate composition of experimental diets (% DM)						
Dry matter	91.6	91.6	91.5	91.5	91.8	92.1
Crude ash	8.86	8.86	8.82	8.80	8.77	8.66
Crude fibre	4.74	4.74	4.52	4.51	5.10	4.29
Crude lipid	11.6	11.6	12.4	12.4	11.9	11.6
Crude protein	30.4	30.4	35.1	35.1	40.1	45.0
GE (KJ g^{-1})	16 7	16 7	17 4	17 4	17 6	17 8

1.Kg^{-1} diet Kg^{-1} Vitamin and Minerals: Vitamin A –10,000,000 I.U.; D3- 2,000,000 I.U.; E –23,000mg; K3 – 2,000 mg; B1 – 3,000 mg; B2- 6,000 mg; Nacin– 50,000 mg; Calcium Pathonate – 10,000 mg; B6 – 5,000 mg; B12- 25.0 mg; Folic acid 1,000 mg; Biotin- 50.0 mg; Choline chloride – 400,000 mg; Manganese – 120,000 mg; Iron- 100,000 mg; Copper– 8,500 mg; Iodine – 1,500 mg; Cobalt-300 mg; Selenium-120 mg; Antioxidant 120,000 mg

Table 2_a. Amino acids composition of the feed ingredients used in the diets

	Corn 10 2% CP	FM 64% CP	SB 44% CP	GNC 49% CP
Lysine	0.26	4.81	2.83	2.67
Histidine	0.25	1.78	1.77	0.96
Arginine	0.40	3.66	3.23	3.33
Threonine	0.30	2.64	1.73	1.65
Methionine	0.18	1.77	0.61	0.65
Cysteine	0.19	0.57	0.70	0.50
Valine	0.42	3.03	2.40	2.45
Isoleucine	0.29	2.57	1.99	1.43
Leucine	1.00	4.54	3.42	3.00
Tyrosine	-	2.04	1.69	1.09
Phenylalanine	0.42	2.51	2.18	1.70

Source: NRC (2011)

Generally, the minerals contents of the fish (Table 5) increased with increasing dietary protein levels or with DL-Met supplementation. Carcass Ca was statistically the same in the fish fed diets 3, 4, 5 and 6, but their values were significantly higher than those in the fish fed diets 1 and 2. Carcass P and Zn followed the same trend as in Ca, while there were no differences (P>0.05) in the Mg contents of the fish. Manganese (Mn) deposition was the same in fish

Table 2$_b$. Amino acids composition of experimental diets (g/100g protein)[1]

	Diet 1 30% CP	Diet 2 30% CP	Diet 3 35% CP	Diet 4 35% CP	Diet 5 40% CP	Diet 6 45% CP
Lysine	1.91	1.91	2.19	2.19	2.47	2.75
Histidine	0.93	0.93	1.05	1.05	1.17	1.28
Arginine	2.08	2.08	2.37	2.37	2.66	2.95
Threonine	1.23	1.23	1.39	1.39	1.56	1.72
Methionine	0.60	0.71	0.68	0.79	0.75	0.83
Cysteine	0.40	0.40	0.44	0.44	0.48	0.51
Valine	1.63	1.63	1.84	1.84	2.06	2.27
Isoleucine	1.22	1.22	1.38	1.38	1.54	1.70
Leucine	2.39	2.39	2.64	2.64	2.90	3.15
Tyrosine	0.91	0.91	1.06	1.06	1.21	1.36
Phenylalanine	1.34	1.34	1.51	1.51	1.67	1.83

1. Data calculated using values from (NRC 2011) nutrient compositions in Table 2$_a$

Table 3. Growth and nutrient utilization of *C. gariepinus* fed the experimental diets

	Diet 1 30% CP	Diet 2 30% CP+Met	Diet 3 35% CP	Diet 4 35% CP+Met	Diet 5 40% CP	Diet 6 45% CP
Initial wt. (g)	2.50	2.40	2.40	2.40	2.40	2.50
Final wt. (g)	24.3	26.4	28.0	37.9	40.4	42.2
Weight gain (g)	$21.8^{d}\pm0.14$	$24.0^{bc}\pm0.34$	$25.7^{b}\pm0.07$	$35.5^{a}\pm0.09$	$38.0^{a}\pm0.05$	$39.7^{a}\pm0.01$
SGR	$3.25^{cd}\pm0.06$	$3.43^{bc}\pm0.10$	$3.51^{b}\pm0.02$	$3.94^{ab}\pm0.09$	$4.03^{a}\pm0.61$	$4.04^{a}\pm0.12$
FCR	$1.16^{a}\pm0.14$	$1.15^{a}\pm0.16$	$1.14^{a}\pm0.20$	$1.14^{a}\pm0.11$	$1.11^{a}\pm0.15$	$1.09^{a}\pm0.17$
PER	$23.3^{a}\pm3.51$	$23.4^{a}\pm4.10$	$23.7^{a}\pm4.67$	$23.8^{a}\pm4.77$	$24.7^{a}\pm4.04$	$26.7^{a}\pm2.08$

Values of 3 replicates on the same row with same superscript are not different (P>0.05)

Table 4. Proximate composition of *C. gariepinus* fed the experimental diets.

	Diet 1 30% CP	Diet 2 30% CP+Met	Diet 3 35% CP	Diet 4 35% CP+Met	Diet 5 40% CP	Diet 6 45% CP
Dry matter	$90.2^{a}\pm1.32$	$90.2^{a}\pm1.32$	$91.0^{a}\pm0.92$	$91.0^{a}\pm0.88$	$91.1^{a}\pm1.76$	$91.2^{a}\pm0.12$
Protein	$56.4^{a}\pm1.32$	$56.4^{a}\pm1.32$	$60.0^{a}\pm0.92$	$60.1^{a}\pm0.88$	$64.3^{a}\pm1.76$	$65.2^{a}\pm0.12$
Lipids	$15.4^{a}\pm0.83$	$15.2^{a}\pm0.83$	$14.6^{a}\pm0.58$	$14.4^{a}\pm0.58$	$13.2^{ab}\pm0.06$	$12.4^{ab}\pm0.56$
Ash	$12.3^{b}\pm0.29$	$12.4^{b}\pm0.31$	$15.3^{a}\pm0.61$	$15.5^{a}\pm0.72$	$15.8^{a}\pm0.96$	$16.1^{a}\pm0.51$
Protein: ash	$4.59^{a}\pm0.39$	$4.55^{a}\pm0.51$	$3.92^{ab}\pm0.67$	$3.90^{ab}\pm0.77$	$4.07^{a}\pm0.26$	$4.05^{a}\pm0.21$

Values of 3 replicates on the same row with same superscript are not different (P>0.05)

fed diets 3, 4, 5 and 6, while fish fed diets 5 and 6 had higher (P<0.05) Mn concentrations than the fish fed diets 1 and 2.

The amino acids profile of the fish (Table 6) shows increasing values with increasing dietary protein levels or with DL-Met supplementation in the diets. This is an indication that supplemented DL-Met aided the synthesis of other amino acids and their deposition in the fish body. Significant differences also existed in the values with increasing protein levels or amino acid supplementation.

Table 5. Minerals composition of *C. gariepinus* fed the experimental diets (mg g^{-1} DM).

	Diet 1 30% CP	Diet 2 30% CP+Met	Diet 3 35% CP	Diet 4 35% CP+Met	Diet 5 40% CP	Diet 6 45% CP
Ca	$18,0^{b}$ ±2.05	18.2^{b}±1.82	32.0^{a}±0.62	32.1^{a}±0.86	34.5^{a}±1.23	36.1^{a}±0.12
P	10.9^{b}±0.16	11.0^{b}±0.21	19.4^{a}±0.05	19.5^{a}±0.58	20.9^{a}±0.36	21.9^{a}±0.05
Mg	3.56^{a}±0.04	3.57^{a}±0.14	3.44^{a}±0.07	3.46^{a}±0.04	3.47^{a}±0.09	3.60^{a}±0.51
Zn	0.16^{b}±0.04	0.16^{b}±0.08	0.19^{a}±0.00	0.20^{a}±0.00	0.29^{a}±0.01	0.32^{a}±0.04
Mn	0.07^{b}±0.06	0.08^{b}±0.10	0.15^{ab}±0.05	0.16^{ab}±0.08	0.20^{a}±0.00	0.25^{a}±0.01

Values of 3 replicates on the same row with same superscript are not different (P>0.05)

Table 6. Amino acids composition in fish carcass (g/100g protein)

Amino acids	Diet 1 30% CP	Diet 2 30% CP+Met	Diet 3 35% CP	Diet 4 35% CP+Met	Diet 5 40% CP	Diet 6 45% CP
Lysine	3.83^{f}±0.01	4.01^{e}±0.01	4.39^{d}±0.01	4.62^{c}±0.01	4.95^{b}±0.02	5.53^{a}±0.03
Histidine	1.86^{f}±0.02	1.95^{e}±0.01	2.10^{d}±0.00	2.22^{c}±0.02	2.34^{b}±0.02	2.57^{a}±0.02
Arginine	4.16^{f}±0.02	4.37^{e}±0.02	4.74^{d}±0.03	5.00^{c}±0.00	5.32^{b}±0.03	5.93^{a}±0.02
Threonine	2.46^{f}±0.02	2.58^{e}±0.02	2.78^{d}±0.02	2.93^{c}±0.01	3.12^{b}±0.02	3.46^{a}±0.02
Methionine	1.20^{e}±0.02	1.49^{c}±0.01	1.36^{d}±0.02	1.50^{c}±0.02	1.56^{b}±0.01	1.67^{a}±0.01
Cysteine	0.80^{e}±0.02	0.84^{d}±0.02	0.88^{c}±0.01	0.93^{b}±0.02	0.96^{b}±0.02	1.03^{a}±0.01
Valine	3.26^{f}±0.01	3.43^{e}±0.02	3.68^{d}±0.02	3.88^{c}±0.02	4.12^{b}±0.01	4.56^{a}±0.02
Isoleucine	2.44^{f}±0.02	2.56^{e}±0.01	2.76^{d}±0.02	2.91^{c}±0.01	3.08^{b}±0.02	3.42^{a}±0.02
Leucine	3.59^{d}±0.01	3.60^{d}±0.02	3.96^{c}±0.01	3.98^{c}±0.03	4.35^{b}±0.02	4.73^{a}±0.02
Tyrosine	1.82^{f}±0.01	1.91^{e}±0.01	2.12^{d}±0.02	2.24^{c}±0.01	2.42^{b}±0.01	2.73^{a}±0.02
Phenylalanine	2.68^{f}±0.02	2.81^{e}±0.01	3.02^{d}±0.02	3.19^{c}±0.01	3.34^{b}±0.03	3.68^{a}±0.02

Values of three numbers on the same row with similar superscripts are not different (P>0.05)

Discussion

Fish require adequate supply of protein for optimum growth and maintenance of physiological function. However, protein is costly and takes between 50 and 70% of the total feed costs (Yang *et al.* 2002; Nwanna 2002) thus confirming it as the single most expensive component of fish feeds (NRC 1993, De Silva and Anderson 1995). Therefore many fish farmers in a bid to reduce feed costs usually produce diets deficient in proteins. This study investigated the effects of such diets on the growth and carcass quality of *C. gariepinus*, and whether supplementation of DL-Met to the protein deficient diets could improve the performance of the fish.

Observation from the present study indicated clearly that inadequate dietary protein retards fish growth and nutrient utilization. Fagbenro et al., (1992) reported the protein requirement of *C. gariepinus*, as 40%, while Adebayo (2005) reported the protein requirement of hybrid catfish as 45%. The results from this study supported the estimation of 40% protein as the requirement for *C. gariepinus* and also showed that increasing the dietary protein content of the fish from 40% to 45% did not have any additional significant advantage. This corroborates the report of Oishi et al. (2010) that excess protein does not support additional increase in growth performance, but rather results in economic losses and deterioration of water quality.

Methionine is a limiting amino acid in many dietary proteins (Poston 1986; NRC, 2011) and its deficiency results in slow growth and reduced feed efficiency in fishes (Cowey et al., 1992). It also plays the role of amino acid balancing protein (Chattopadhyay et al. (2006). Therefore the improvement and similar growth performance of the fish fed 35% protein diet supplemented with Met compared with those fed diets with 40 or 45% protein diets could be because supplemental Met increased the Met content in that diet to be close with Met in diets with 40 or 45% protein. Williams *et al*. (2001) demonstrated that crystalline-EAA supplementation to an EAA deficient diet increased fish growth and the response was more pronounced for the low protein diets. Li and Robinson (1998) and Yamamoto *et al*. (2005), showed that supplementation of EAA in protein deficient diet improved the feed efficiency but did not enhance the growth of channel catfish (*Ictalurus punctatus)* and rainbow trout (*Oncorhynchus mykiss*), respectively. Observations in this study also agreed with those of Kerr et al. (2003) that pigs fed 12% CP diet supplemented with crystalline amino acid had similar weight gain and carcass characteristics with other group fed 16% CP diet. The findings on growth performance of the fish from the present study is in accordance with the report of Nwanna et al. (2012) that supplementation of DL-Met to a diet improved final weight, daily weight gain, feed efficiency and protein digestibility in common carp (*Cyprinus carpio)*.

Similarly, research findings have revealed improved growth performance with supplementation of crystalline amino acids to amino acid-deficient proteins in practical diets for fishes (Robinson, 1991; Schwarz *et al.,* 1998; Mukhopadhyay and Ray, 1999). Pesti (2009) demonstrated increase in growth and feed utilization efficiency of broiler chickens with increasing levels of dietary protein- a clear indication that the ideal ratios of the essential amino acids in the diets is maintained. The results from the present study strongly support this view as there was corresponding increase in the growth of the fish with increasing dietary protein supply.

In the present study, carcass protein and ash contents of the fish were positively correlated with dietary protein levels or with Met supplementation, while the lipid content of the fish had inverse relationship with dietary protein levels or Met supplementation. Similar results on carcass body protein of other species of fish have been reported (Gunasekera *et al*., 2000; Liebert *et al*., 2006). The results of the present study are in strong agreement with earlier findings by Schwarz *et al*. (1998) of increased body protein and decreased body fat content in common carp with increasing dietary methionine supplementation. Sardar *et al*. (2009) also reported increased nitrogen retention and decreased lipid retention in Indian major carp with both supplemental Met and lysine and additive effects using a combination of both amino acids.

Nwanna *et al.* (2012) similarly reported that supplemental DL-Met raised the dietary methionine content resulted in significant increase in carcass protein that likely improved carcass quality. Further more, the report of Ozoro *et al*. (2002) showed that dietary lysine supplements markedly improved protein retention efficiency, measured as net protein utilization (NPU), in *C. gariepinus*.

Another aspect of lipid retention and methionine interrelationship is that Met may act as lipotropic agent (Chattopadhyay *et al.,* 2006). Chen *et al.* (1993) and Chattopadhyay *et al.* (2006) explained that the ability of methionine to act as lipotropic agent is either because of its role as an amino acid balancing protein or through its role as a methyl donor and involvement in or link to choline, betaine, folic acid and vitamin B_{12} metabolism. The observed inverse relationship between the carcass lipid and dietary protein levels has also been noted with other fish species (Khan *et al.* 1993; Chen and Tsai 1994; Arzel *et al*. 1995; Yang *et al.* 2002; Oishi et al., 2010). However, this observation is in contrast with that of Shiau and Lan (1996) who reported positive correlation between carcass lipid of grouper (*Epinephelus malabaricus)* and dietary protein levels. They also opined that differences in carcass lipid content might be attributed to differences in the dietary carbohydrates as fish differ in their ability to utilize carbohydrates.

The mineral composition of the experimental fish explains the increasing levels of carcass ash with increasing levels of protein or amino acid supplementation in the diets. The calcium and phosphorus contents of the fishes made up about 80 - 90% of the total minerals thus making them the dominant inorganic components in the whole fish body, as previously described by (Skonberg et al., 1997, Hertrampf and Piedad-Pascual, 2000). Skeletal mineralization affects the level of ash and hence the mineral contents in animals (Clawson et al. 1991). The hypothesis that protein ash ratio in birds, mammals and fishes is constant was not well demonstrated in the present study as the ratio obtained was not constant and without any sound explanation. This is in line with the documentation of Clawson et al. (1991) which stated that the protein: ash ratio in the whole body of representative mammals, birds and fishes is not constant but subject to effects of nutrition, physiological state, sex, genetics and age of the animal. They further explained that, in some cases of amino acids or protein deficiency, the protein: ash ratio increased relative to the control because the dietary Ca or P intake or absorption limited skeletal mineralization, which is in line with the results obtained from the present study by feeding the fish with 30% CP diet (diet 1).

However, this area of study needs further investigation to establish how skeletal development and protein: ash ratio in the body of animals is regulated.

The free amino acid content in the blood plasma initially reflects the content of the amino acids in the dietary protein following absorption (Blasco et al., 1991). The increasing levels of amino acids due to increasing protein levels observed from the study supports this assertion. Similarly, the corresponding increase in other amino acids in the fish carcass apart from Met following Met supplementation in the diets has been reported by some researchers. While others' reported reduction of certain amino acids in fish plasma in response to raising certain others. Our observation is in consonance with the report of Schwarz et al., 1998 and Nwanna et al. (2012) which showed that supplemented DL-Met significantly increased the plasma methionine and arginine contents in *C. carpio*. Murai et al. (1989) found significantly lower Met content in plasma of carp fed a diet of soya flour 6 h after feeding, which increased clearly when L-Met was added. The increase in the plasma Met level supports the work of Simmons et al. (1999) who also reported improved plasma Met levels in Arctic charr *Salvelinus alpinus*. as a result of Met supplementation in the diets. Similarly, Kwasek et al. (2009) demonstrated that supplementation of peptide- and free amino acids in Silver bream (*Vimba vimba)* diets increased the taurine levels in the body which corresponded to observed weight gains. Also Kim *et al.* (2005) reported that supplementation of 1% taurine to diet increased body weight gain and the corresponding taurine levels in the muscle and liver of Japanese flounder (*Paralichthys olivaceus).* Our observation that supplementation of Met in the diets increased other amino acids in the fish carcass is supported by Nordrum *et al. (*2000) that supplementation of cysteine in the diet caused a marked increase in glycine and glutamate levels of Atlantic salmon (*Salmo salar)*. They also reported that supplementation of cysteine and Met in the diet caused elevation of plasma taurine and suggested that the fish may have the ability to effectively convert methionine to taurine. However, contrary to our observation, Kaushik and Fauconneau (1984) reported decrease in arginine in plasma of *O. mykiss* in response to raising dietary lysine level. Murai *et al*. (1989) and Nwanna *et al.* (2012) also reported that surprisingly, the lysine and serine contents in plasma of fish fed Met deficient diet were more than in the fish fed diet adequate in Met and they concluded that higher content of amino acids in the plasma of fish fed Met deficient diets may be connected with a depressed protein synthesis. Similarly, Nordrum *et al.* (2000) reported that alanine supplementation in diet did not even affect plasma alanine concentration in contrast to the elevation observed for plasma methionine with the Met supplemented diet. In another development, De-Lange *et al.* (2001) described that dietary Thr intake did not influence the Thr content of growing pigs fed purified diets.

Conclusion

In conclusion, results from this study, suggests that when the dietary energy requirement of a fish is maintained, that adequate dietary protein will provide sufficient amino acids required by that fish. Also, the data confirmed the protein requirement of the fish to be 40%. However 35% crude protein diet supplemented with 0.11% Met was as efficient as a diet of 40% CP in supporting the growth performance and nutrient utilization.

References

Adebayo, T. (2005). Dietary protein level and feeding rate for hybrid Clariid catfish, *Clarias gariepinus* × *Heterobranchus bidorsalis*, in homestead tanks. *Journal of Applied Aquaculure* 9(1), 97-106.

A.O.A.C. (Association of Official Analytical Chemists - A.O.A.C). (1995). *Official methods of analysis*. 12th Edition. George Banta Co. Inc. Manasha, Winsconsin, USA. 937 p.

Arzel, J., Metailler, R., Kerleguer, C., Le Delliou, H. & Guilaume, J. (1995). The protein requirement of brown trout (*Salmo trutta*) fry. *Aquaculture* 130, 67-78.

Blasco J., Fernandez J. and J. Gutierrez, 1991. The effects of starvation and re-feeding on plasma amino acid levels in carp, *Cyprinus carpio*.(L.) *Journal of Fish Biology* 38, 587–598.

Chattopadhyay, K., Mondal, K. & Roy, B. (2006). Comparative efficacy of DL-methionine and herbal methionine on performance of broiler chicken. *International Journal of Poultry Science* 5, 1034–1039.

Chen F., Noll, L., Waibel, E. & Hawkins, D. (1993). Effect of folate, vitamin B12 and choline supplementation on turkey breeder performance. *Poultry Science* 72, 73–77.

Chen, Y. & Tsai, C. (1994). Optimal dietary protein level for the growth of juvenile grouper, *Epinephelus malabaricus*, fed semipurified diets. *Aquaculture* 119, 265-271.

Clawson, A., Garlich, J., Coffey, M. & Pond, W. (1991). Nutritional, physiological, genetic, sex, and age effects on fat-free dry matter composition of the body in avian, fish and mammalian species: a review. *Journal of Animal Science* 69 (9), 3617-44.

Cowey, C., Cho, C., Sivak, J., Weerheim, J. & Stuart, D. (1992). Methionine intake in rainbow trout (Oncorhynchus mykiss) relationship to cataract formation and the metabolism of methionine. *Journal of Nutrition* 122, 1154-1163.

De Lange, C., Gillis, A. & Simpson, G. (2001). Influence of threonine intake on whole-body protein deposition and threonine utilization in growing pigs fed purified diets. *Journal of Animal Science* 79(12), 3087-95.

De Silva, S. & Anderson, T. (1995). *Fish Nutrition in Aquaculture*. Chapman & Hall, London, UK, 319 pp.

Duncan, D. (1955). *Multiple range and multiple (F) test. Biometrics* 11, 1-42.

Hertrampf, J. & Piedad-Pascual, F. (2000). Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Publishers, the Netherland. 573 pp.

Fagbenro, O., Balogun, A. & Anyanwu, C. (1992). Optimum dietary protein level of *Heterobranchus bidorsalis* fed compounded diet. *Nigerian Journal of Applied Fish Hydrobiology* 1, 41-45.

FAO, (2012). *The state of the world fisheries and aquaculture, 2012. www.fao.org/icatalog/inter-e.htm*

Gunasekera, R., De Silva, S., Collins, R., Gooley G. & Ingram, B. (2000). Effect of dietary protein level on growth and food utilization in juvenile Murray cod *Maccullochella peelii* (Mitchell). *Aquaculture Research* 31, 181-187.

Kaushik, S. & Fauconneau, B. (1984). Effects of lysine administration on plasma arginine and some nitrogenous catabolites in rainbow trout. *Comperative Biochemistry and Physiology* 79, 459–462.

Kendall, P. (2003). Turning carnivorous fish into vegetarians. *Nature* 426, 379- 379. www.nature.com/nature

Kerr, B., Southern, L., Bidner, T., Friesen, K. & Easter, R. (2003). Influence of dietary protein level, amino acid supplementation, and dietary energy levels on growing-finishing pig performance and carcass composition. *Journal Animal Science* 81(12), 3075-87.

Khan, C.; Ang, K., Ambak, M. & Saad, C. (1993). Optimum dietary protein requirement of a Malaysian freshwater catfish, *Mystus nemurus*. *Aquaculture*, 112, 227-235.

Kim, S., Takeuchi, K., Akimoto, A., Furuita, A.,Yamamoto, T., Yokoyama, M. & Murata, Y. (2005). Effect of taurine supplemented practical diet on growth performance and taurine contents in whole body and tissues of juvenile Japanese flounder, *Paralichthys olivaceus*. *Fisheries*

Science 71, 627-632.

Kwasek, K., Zhang,Y., Hliwa, P., Gomuzka, P., Ostaszewska, T. & Dabrowski, K. (2009). Free amino acids as indicators of nutritional status of silver bream (*Vimba vimba*), when using commercial and purified diet. *Comperative Biochemistry and Physiology* Part A 153, 113-119.

Li, M. & Robinson, H. (1998). Effects of supplemental lysine and methionine in low protein diets on weight gain and body composition of young channel catfish Ictalurus punctatus. *Aquaculture* 163, 297-307.

Liebert, F., Sünder, A. & Mohamed, K (2006). Assessment of nitrogen maintenance requirement and potential for protein deposition in juvenile tilapia genotypes by application of an exponential nitrogen utilization model. *Aquaculture* 261, 1346–135.

Lovell, T. (1989). Diet and fish nutrition husbandry. In: Halver JE (ed) Fish Nutrition. Academic Press, California, London, pp 549–604.

Luo, Z., Liu, Y., Mai, K., Tian, L., Liu, D. & Tian, X. (2004). Optimal dietary protein requirement of grouper *Epinepheluscoioides* juveniles fed iso-energetic diets in floating net cages. *Aquaculture Nutrition* 10, 247–252.

Mohapatra, S., Chakraborty, A., Prusty, K., Das, P., Paniprasad, K. & Mohanta, K. (2012). Use of different microbial probiotics in the diet of rohu, *Labeo rohita* fingerlings: effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. *Aquaculture Nutrition* 18, 1-11.

Mukhopadhyay, N. & Ray, A. (1999). Improvement of quality of sesame *Seasamum indicum* seed meal protein with supplemental amino acids in feeds for rohu, *Labeo rohita* (Hamilton) fingerlings. *Aquaculture Resources* 3, 549–557.

Murai, T., Wang, D. & Ogata, H. (1989). Suplementation of methionine to soy flour diets for fingerling of carp, *Cyprinus carpio*. *Aquaculture* 77, 373–385.

Nordrum, S., Krogdahl, A., Røsjø, C., Olli, J. & Holm, H. (2000). Effects of methionine, cysteine and medium chain triglycerides on nutrient digestibility, absorption of amino acids along the intestinal tract and nutrient retention in Atlantic salmon (*Salmo salar* L) under pair-feeding regime. *Aquaculture* 186, 341–360.

NRC, (National Research Council). (1993). *Nutrient requirements of fish,* Washington, DC, USA, National Academic Press, Washington, DC, 114 pp.

NRC, (National Research Council). (2011). *Nutrient requirements of fish and shrimp,* Washington, DC, USA, National Academic Press, Washington, DC, 376 pp.

Nwanna, L. (2002). Performance of hybrid clariid catfish fingerlings (male *Heterobranchus Bidorsalis x* female *Clarias gariepinus*) fed poultry layer waste diets in glass tanks. *Journal of Applied Aquaculture* 12(3), 99-106.

Nwanna, L., Lemme, A., Metwally, A. & Schwarz, F. (2012). Response of common carp (Cyprinus carpio L.) to supplemental DL-methionine and different feeding strategies *Aquaculture* 356–357 (2012): 365–370.

Oishi, C., Nwanna, L. & Manoel, P. (2010). Optimum dietary protein requirement for Amazonian Tambaqui (*Colossoma macropomum* Cuvier, 1818) fed fish meal free diets. ACTA AMAZONICA 40(4), 757-762.

Ozoro, R., Booms, O., Huisman, G. & Verreth, J. (2002). Changes in amino acid composition in the tissues of African catfish (*Clarias gariepinus*) as a consequence of dietary L-carnitine supplements. *Journal of Applied Ichthyology* 18, 140–147.

Pesti, G. (2009). Impact of dietary amino acid and crude protein levels in broiler feeds on biological performance. *Journal of Applied Poultry Resources* 18, 477-486.

Poston, H. (1986). Response of rainbow trout to source and level of supplemental dietary methionine. *Comparative Biochemistry and Physiology* 83A, 739-744.

Robinson, E. (1991). Improvement of cotton seed meal protein with supplemental lysine in feeds for channel catfish. *Journal of Applied Aquaculture* 1, 1–14.

Sardar, P., Abid, M., Randhawa, H. & Prabhakar, S. (2009). Effect of dietary lysine and methionine supplementation on growth, nutrient utilization, carcass compositions and haemato-biochemical status in Indian Major Carp, Rohu (*Labeo rohita* H.) fed soy protein-based diet. *Aquaculture Nutrition* 15, 339–346.

SAS, (2003). Statistical Analysis Software (SAS) systems for Windows, SAS Inc., Cary. NC, USA.

Schwarz, F., Kirchgeßner, M. & Deuringer, U. (1989). Studies on the methionine requirement of carp (*Cyprinus carpio* L.). *Aquaculture* 161, 121–129.

Shiau, S. & Lan, C. (1996). Optimum dietary protein level and protein to energy ratio for growth of grouper (*Epinephelus malabaricus*). *Aquaculture* 145, 259-266.

Simmons, L., Moccia, R. & Bureau, D. (1999). Dietary methionine requirement of juvenile Arctic charr, *Salvelinus alpinus* (L). *Aquaculture Nutrition* 5, 93–100.

Skonberg, D., Yogev, L., Hardy, R. & Dong, F. (1997). Metabolic response to dietary phosphorus intake in rainbow trout *(Oncorhynchus mykiss).* Aquaculture 157,11-24.

Sugiura, S., Gabaudan, J., Dong, F. & Hardy, R. (2001). Dietary microbial phytase supplementation and the utilization of phosphorus, trace minerals and protein by rainbow trout (*Oncorhynchus mykiss*) (Walbaum) fed soybean meal-based diets. *Aquaculture Research* 32, 583-592.

Williams, K., Barlow, C. & Rodgers, L. (2001). Efficacy of crystalline and protein-bound amino acids for amino acid enrichment of diets for *barramundi/Asian seabass (Lates calcarifer).* Aquaculture Research 1, 415-429.

Yamamoto, T., Sugita, T. & Furuita, H. (2005). Essential amino acid supplementation to fish meal-based diets with low protein to energy ratios improves the protein utilization in juvenile rainbow trout, *Oncorhynchus mykiss.* Aquaculture 246, 379–391.

Yang, S., Liou, C. & Liu, F. (2002). Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). *Aquaculture* 213, 363-372.

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